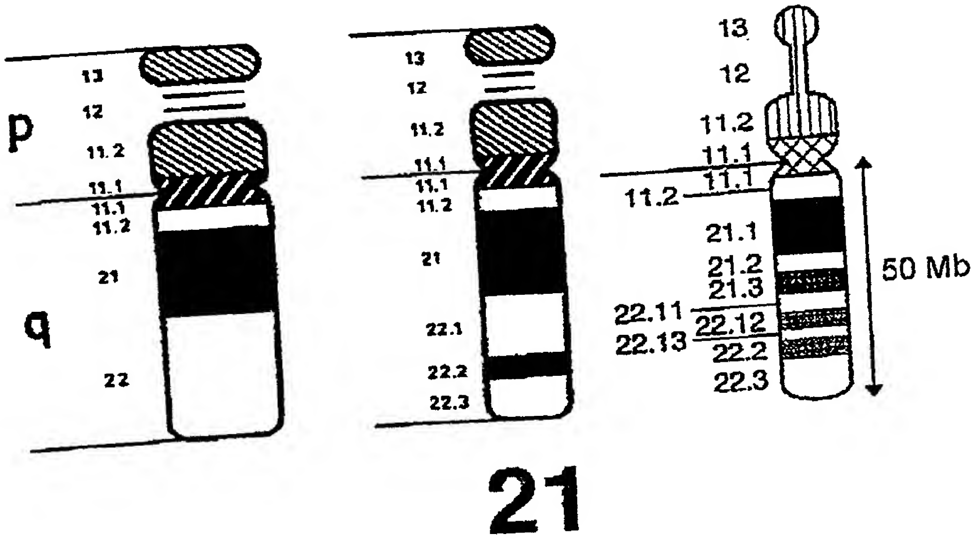

p
q
13
12
11.2
11.1
11.1
11.2
21
22
13
12
11.2
11.1
11.1
11.2
21
22.1
22.2
22.3
13
12
11.2
11.1
11.1
11.2
21.1
21.2
21.3
22.11
22.12
22.13
22.2
22.3
50 Mb
21

Figure 1

Title: METHODS, SOFTWARE AND APPARATI FOR
IDENTIFYING GENOMIC REGIONS HARBORING A
GENE ASSOCIATED WITH A DETECTABLE TRAIT
Inventors: Nicholas SCHORK, et al.
Assignee: Genset Corporation
Our Ref.: 55.US4.DIV
2/31

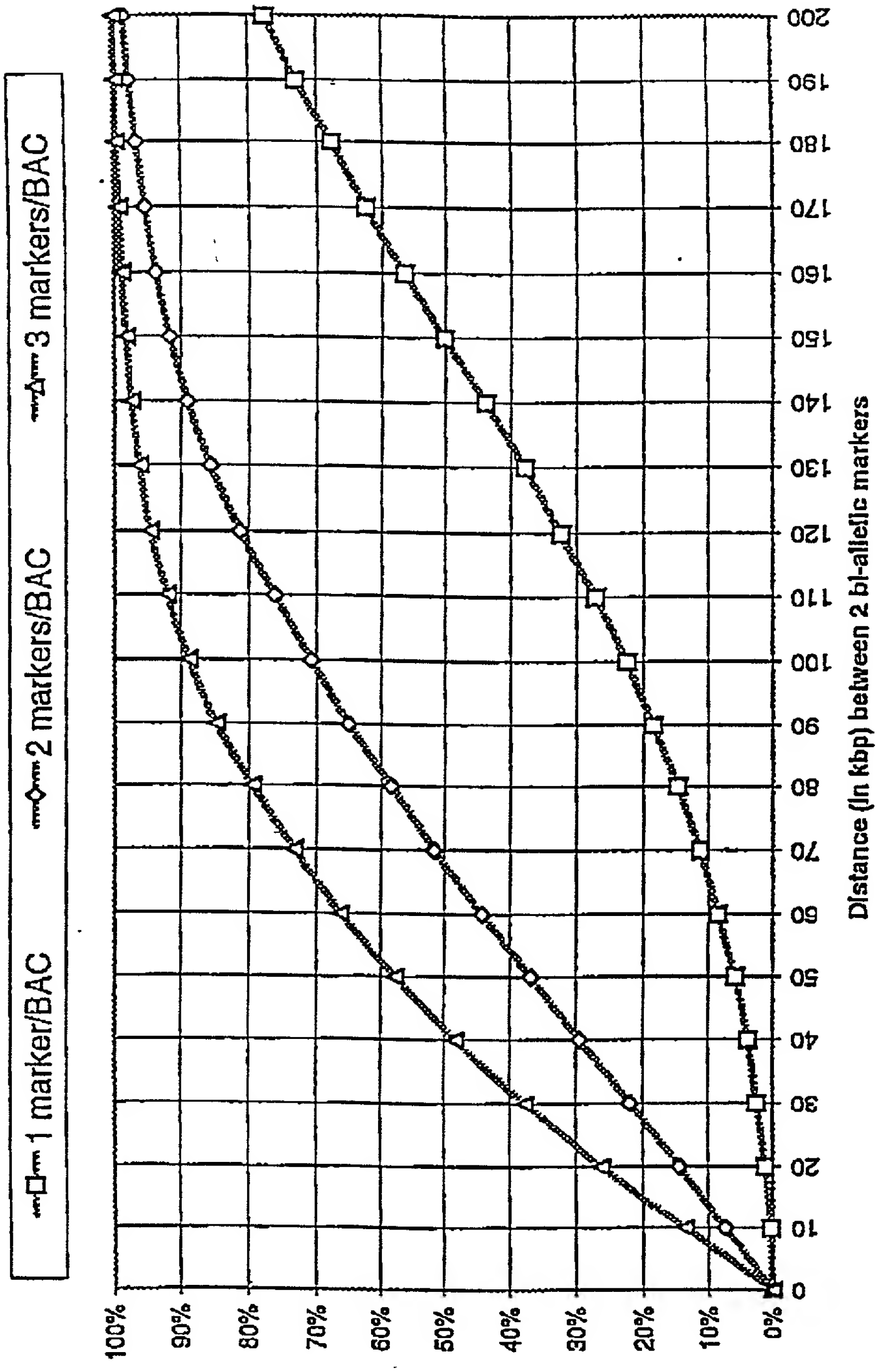


Figure 2A

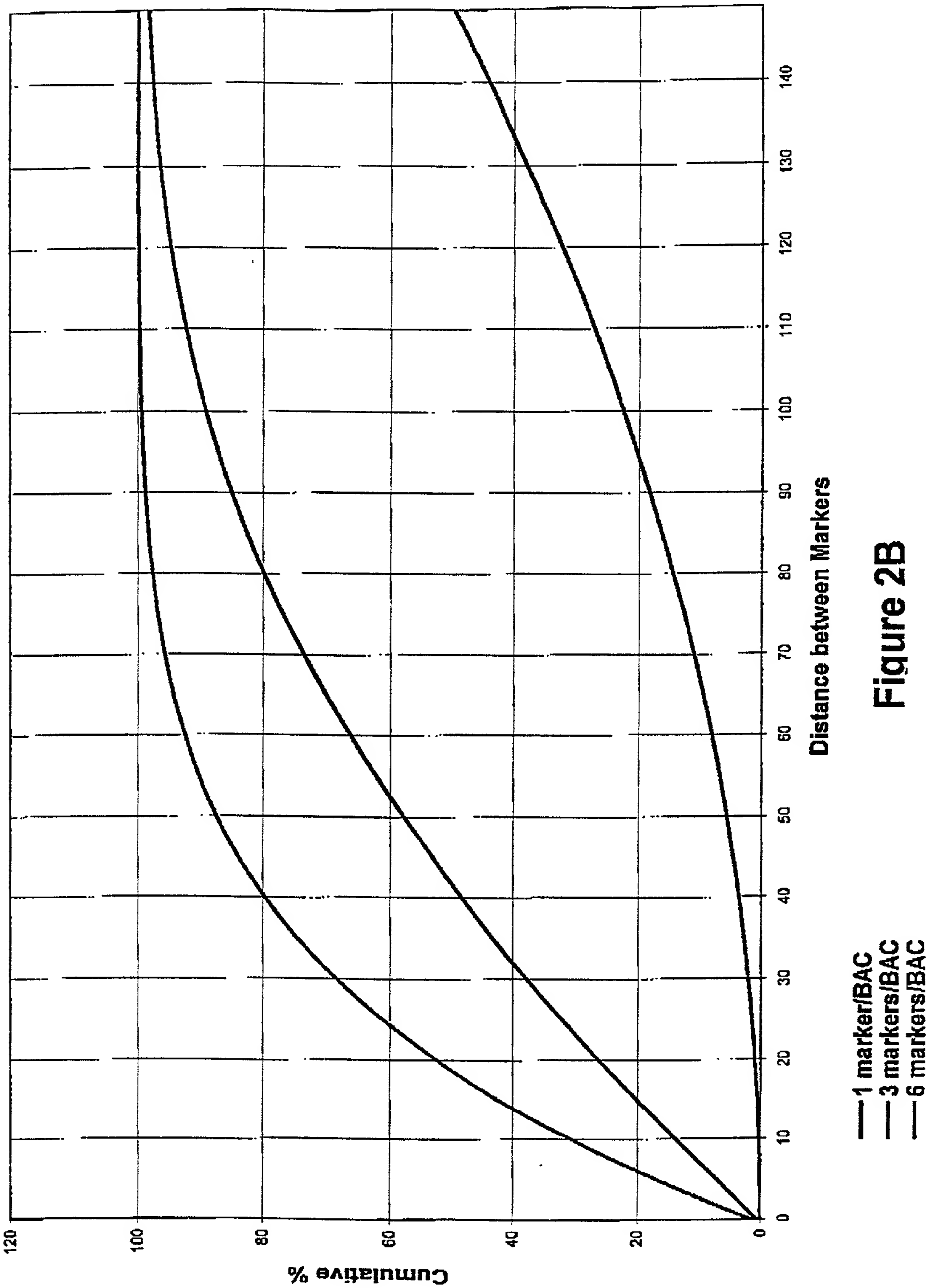
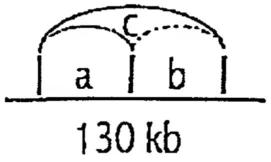

Cumulative %
0
20
40
60
80
100
120
Distance between Markers
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
1 marker/BAC
3 markers/BAC
6 markers/BAC

Figure 2B

LD in a random French caucasian population

- 54 sized « random » BACs covering 8100 kb
- 213 SNP ; 2 to 6 / BAC, mean allele frequency = 0.3
- Order and distance unknown
- For 1 BAC :



* $\overline{m}$ intermarker distance : 130/3 = 43 kb

* $\overline{m}$ LD strength estimate : m (a,b,c) = 0.51

- For 54 BACs :

* $\overline{m}$ intermarker distance = 38 kb

* $\overline{m}$ LD strength estimate = 0.63 ± 0.05
(324 pairs)

- For 19 unlinked SNPs : m LD strength estimate = 0.12 ± 0.007
(171 pairs)

Figure 2c

p-VALUE DISTRIBUTION

# aff	150						
# non aff	150						
	pAi non aff	0	0,1	0,2	0,3	0,4	0,5
Δ pAi	0,05	8,77E-05	0,06407752	0,14252002	0,19106311	0,21543442	0,22009395
Δ pAi	0,1	1,91E-08	0,00060364	0,00467774	0,01023571	0,01382303	0,01382303
Δ pAi	0,15	3,06E-12	1,3319E-06	3,8827E-05	0,0001478	0,0002343	0,00020218
Δ pAi	0,2	3,22E-16	9,1413E-10	9,0305E-08	5,733E-07	9,6336E-07	5,733E-07
Δ pAi	0,25	2,08E-20	2,2614E-13	6,2679E-11	5,873E-10	8,7113E-10	2,5396E-10
Δ pAi	0,3	7,82E-25	2,152E-17	1,3261E-14	1,5189E-13	1,5189E-13	1,3261E-14
Δ pAi	0,35	1,62E-29	7,9823E-22	8,4152E-19	9,1669E-18	4,2713E-18	5,5844E-20
Δ pAi	0,4	1,73E-34	1,1282E-26	1,524E-23	1,1488E-22	1,524E-23	1,1282E-26

# aff	200						
# non aff	200						
	pAi non aff	0	0,1	0,2	0,3	0,4	0,5
Δ pAi	0,05	5,92E-06	0,03250945	0,09039173	0,13111935	0,15260313	0,15678006
Δ pAi	0,1	8,65E-11	7,4765E-05	0,00109084	0,00302686	0,00447365	0,00447365
Δ pAi	0,15	8,02E-16	2,3653E-08	2,0257E-06	1,1771E-05	2,1573E-05	1,7772E-05
Δ pAi	0,2	4,18E-21	1,5375E-12	6,7374E-10	7,764E-09	1,5417E-08	7,764E-09
Δ pAi	0,25	1,13E-26	2,525E-17	4,4025E-14	8,5532E-13	1,4423E-12	2,8149E-13
Δ pAi	0,3	1,47E-32	1,1488E-22	5,8424E-19	1,4886E-17	1,4886E-17	5,8424E-19
Δ pAi	0,35	8,62E-39	1,4784E-28	1,5457E-24	3,6958E-23	1,3394E-23	4,197E-26
Δ pAi	0,4	2,09E-45	5,2308E-35	7,6438E-31	1,1224E-29	7,6438E-31	5,2308E-35

# aff	affected individuals
# non aff	non affected individuals
pAi non aff	allele frequency in non affected individuals
Δ pAi	% Difference in allele frequency between affected and non-affected individuals

Figure 3 (I)

p-VALUE DISTRIBUTION

# aff	500						
# non aff	500						
	pAi non aff	0	0,1	0,2	0,3	0,4	0,5
Δ pAi	0,05	8E-13	0,00072323	0,00741965	0,0169842	0,02371865	0,02516449
Δ pAi	0,1	1,07E-24	3,7948E-10	2,4176E-07	2,7579E-06	6,9679E-06	6,9679E-06
Δ pAi	0,15	3,81E-37	1,0719E-18	5,8344E-14	4,2622E-12	1,8601E-11	1,1611E-11
Δ pAi	0,2	2,96E-50	5,0895E-29	1,6881E-22	6,9321E-20	3,7441E-19	6,9321E-20
Δ pAi	0,25	4,27E-64	7,2043E-41	7,7528E-33	1,194E-29	4,3462E-29	7,6438E-31
Δ pAi	0,3	9,7E-79	3,9328E-54	6,3017E-45	1,9429E-41	1,9429E-41	6,3017E-45
Δ pAi	0,35	2,91E-94	8,8513E-69	8,7879E-59	2,3478E-55	1,8839E-56	1,1206E-62
Δ pAi	0,4	9,5E-111	7,7199E-85	1,8063E-74	1,4484E-71	1,8063E-74	7,7199E-85

# aff	150						
# non aff	850						
	pAi non aff	0	0,1	0,2	0,3	0,4	0,5
Δ pAi	0,05	2,16E-20	0,00994614	0,04896055	0,08358651	0,10417953	0,11025423
Δ pAi	0,1	2,01E-39	5,571E-07	0,00010149	0,00058665	0,00119145	0,00139743
Δ pAi	0,15	1,11E-58	2,7555E-13	8,462E-09	2,9851E-07	1,2395E-06	1,6229E-06
Δ pAi	0,2	3,27E-78	2,1683E-21	3,2211E-14	1,1049E-11	1,111E-10	1,5638E-10
Δ pAi	0,25	4,96E-98	4,4952E-31	6,5226E-21	3,1015E-17	2,5169E-16	1,1763E-15
Δ pAi	0,3	3,7E-118	3,6987E-42	8,129E-29	6,9335E-24	5,4331E-22	6,5657E-22
Δ pAi	0,35	1,4E-138	1,6797E-54	7,1058E-38	1,2938E-31	2,9415E-29	2,5869E-29
Δ pAi	0,4	2,4E-159	5,4915E-68	4,8846E-48	2,1003E-40	1,3332E-37	6,8178E-38

# aff	affected individuals
# non aff	non affected individuals
pAi non aff	allele frequency in non affected individuals
Δ pAi	% Difference in allele frequency between affected and non-affected individuals

Figure 3 (II)

p-VALUE DISTRIBUTION

# aff	200						
# non aff	500						
	pAi non aff	0	0,1	0,2	0,3	0,4	0,5
Δ pAi	0,05	1,06E-12	0,00789803	0,03942584	0,06867566	0,08621572	0,09083704
Δ pAi	0,1	3,45E-24	4,4217E-07	5,6883E-05	0,00031976	0,0006363	0,00070881
Δ pAi	0,15	5,9E-36	4,3025E-13	3,3635E-09	9,2134E-08	3,319E-07	3,5871E-07
Δ pAi	0,2	4,73E-48	1,5566E-20	1,0346E-14	1,7218E-12	1,1512E-11	1,0047E-11
Δ pAi	0,25	1,67E-60	3,5436E-29	2,0473E-21	2,2178E-18	1,1498E-17	1,3524E-17
Δ pAi	0,3	2,46E-73	7,2498E-39	3,0748E-29	2,0601E-25	3,4525E-24	7,4807E-25
Δ pAi	0,35	1,44E-86	1,6945E-49	3,9559E-38	1,4118E-33	2,662E-32	1,4118E-33
Δ pAi	0,4	3,2E-100	5,3051E-61	4,7325E-48	7,1282E-43	1,0691E-41	7,2652E-44

# aff	500						
# non aff	1000						
	pAi non aff	0	0,1	0,2	0,3	0,4	0,5
Δ pAi	0,05	6,48E-24	5,7827E-05	0,00172627	0,00551541	0,00882876	0,00978249
Δ pAi	0,1	6,53E-47	3,065E-14	1,0301E-09	4,3205E-08	1,8833E-07	2,2731E-07
Δ pAi	0,15	1,2E-70	2,0716E-27	3,7441E-19	4,6626E-16	6,9719E-15	6,9719E-15
Δ pAi	0,2	3,33E-95	1,1636E-43	1,6614E-31	8,5632E-27	4,1421E-25	1,9885E-25
Δ pAi	0,25	1,2E-120	1,7683E-62	1,5329E-46	3,1722E-40	8,6765E-39	3,6071E-39
Δ pAi	0,3	5,3E-147	1,526E-83	4,2697E-64	2,5968E-56	3,9328E-54	2,5968E-56
Δ pAi	0,35	2,4E-174	1,184E-106	4,5658E-84	4,7426E-75	4,2624E-73	4,0958E-77
Δ pAi	0,4	9,4E-203	1,082E-131	2,137E-106	1,8014E-96	3,3252E-95	6,725E-102

# aff	affected individuals
# non aff	non affected individuals
pAi non aff	allele frequency in non affected individuals
Δ pAi	% Difference in allele frequency between affected and non-affected individuals

Figure 3 (III)

Title: METHODS, SOFTWARE AND APPARATI FOR IDENTIFYING GENOMIC REGIONS HARBORING A GENE ASSOCIATED WITH A DETECTABLE TRAIT
Inventors: Nicholas SCHORK, et al.
Assignee: Genset Corporation
Our Ref.: 55.US4.DIV
8/31

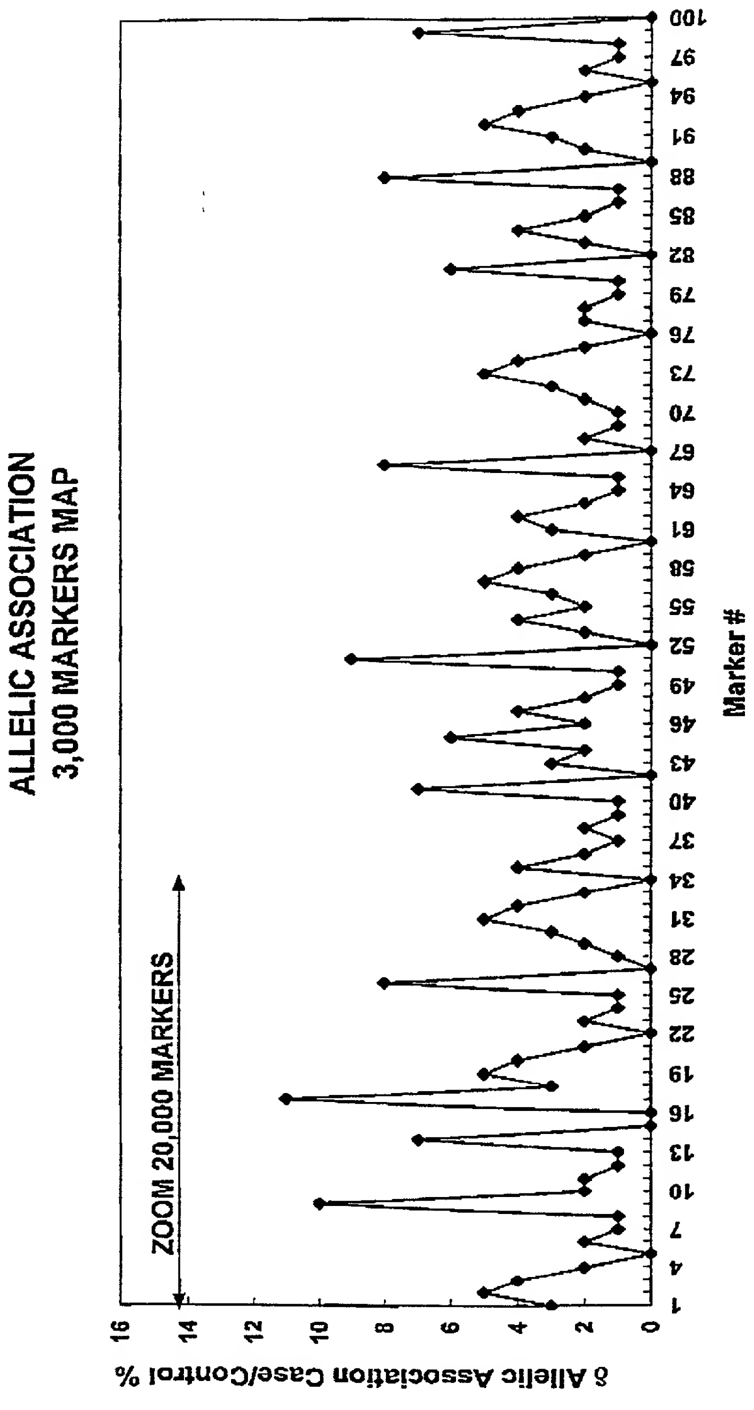


Figure 4

Title. METHODS, SOFTWARE AND APPARATI FOR
IDENTIFYING GENOMIC REGIONS HARBORING A
GENE ASSOCIATED WITH A DETECTABLE TRAIT
Inventors: Nicholas SCHORK, et al.
Assignee: Genset Corporation
Our Ref.: 55.US4.DIV

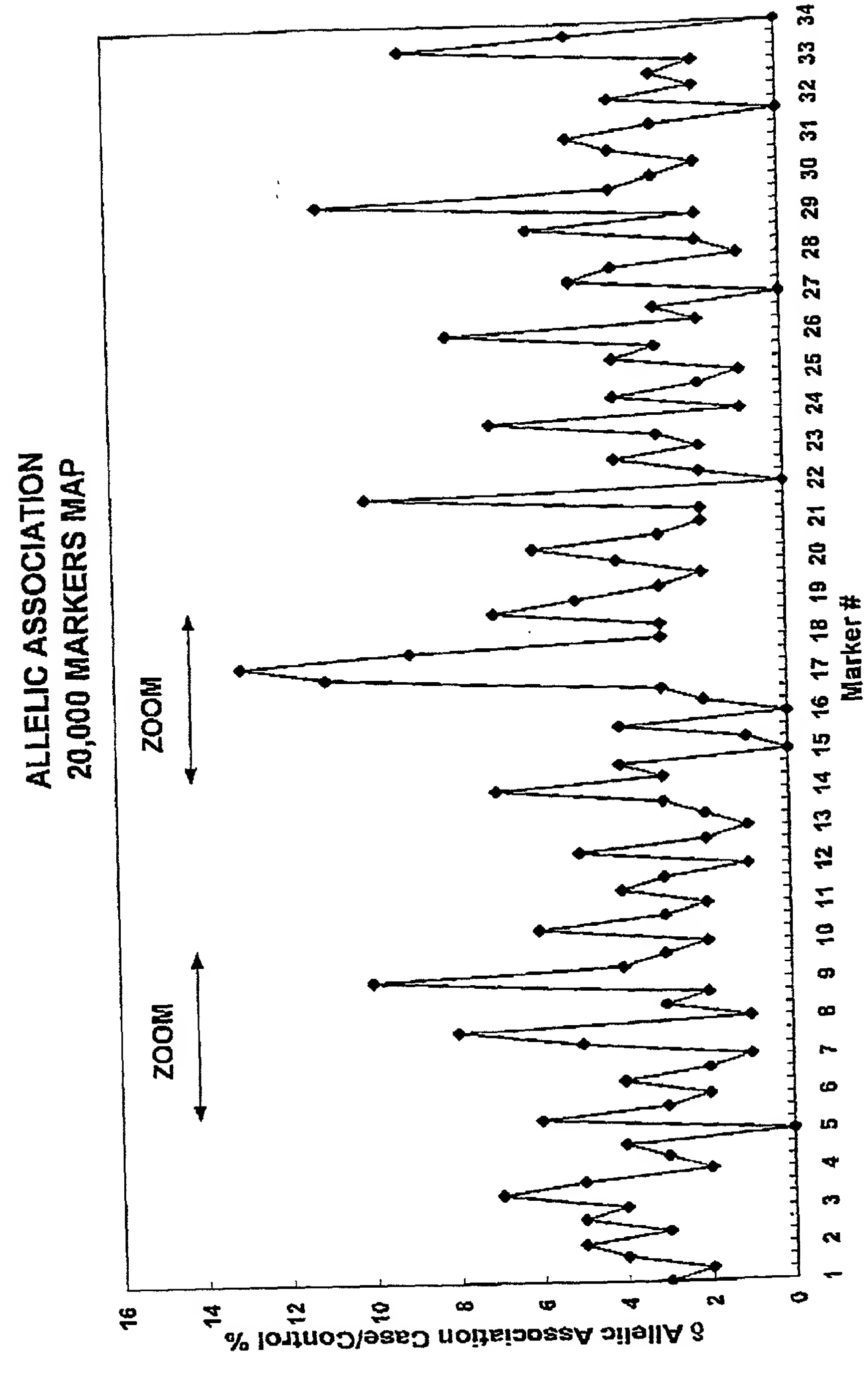


Figure 5

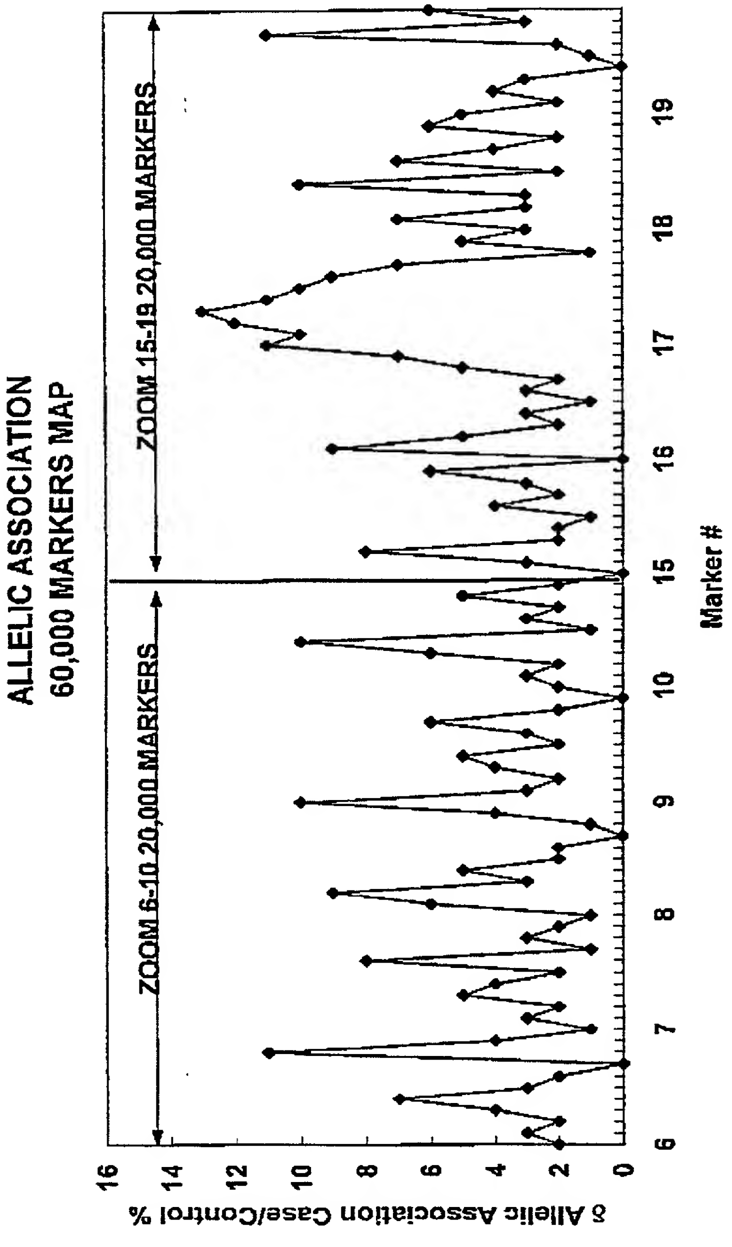

ALLELIC ASSOCIATION
60,000 MARKERS MAP
ZOOM 6-10 20,000 MARKERS
ZOOM 15-19 20,000 MARKERS
δ Allelic Association Case/Control %
16
14
12
10
8
6
4
2
0
6
7
8
9
10
15
16
17
18
19
Marker #

Figure 6

APO E REGION HAPLOTYPE FREQUENCY ANALYSIS

POPULATIONS	AD CASES (225)	AD CONTROLS (248)

markers	99-366	99-344	99-359	99-355	haplotype frequencies		odds-ratio	P value
					cases	controls		
p value	3,01E-01	1,11E-01	6,63E-01	1,38E-01	0,404	0,308	1,52	3,05E-03 ***
haplotype 1	C	G			0,203	0,165	1,29	1,24E-01 *
haplotype 2		G	A		0,375	0,306	1,36	2,83E-02 **
haplotype 3			G	G	0,264	0,209	1,36	5,95E-02 **
haplotype 4	C		A		0,115	0,071	1,70	1,64E-02 **
haplotype 5		G		A	0,15	0,129	1,19	3,59E-01 *
haplotype 6	C			A	0,225	0,122	2,09	4,76E-05 *****
haplotype 7	T		G	G	0,228	0,108	2,44	2,05E-06 ******
haplotype 8	T	A	G	G				

Figure 7

APO E REGION HAPLOTYPE SIMULATION
POPULATION : 225 CASES vs 248 CONTROLS

	4 Markers				haplotype frequencies		odds-ratio	pvalue
	99-344/439	99-366/274	99-359/308	99-355/219	cases	controls		
Haplotype 8	A	T	G	G	0,228	0,108	2,44	2,05E-06 ******

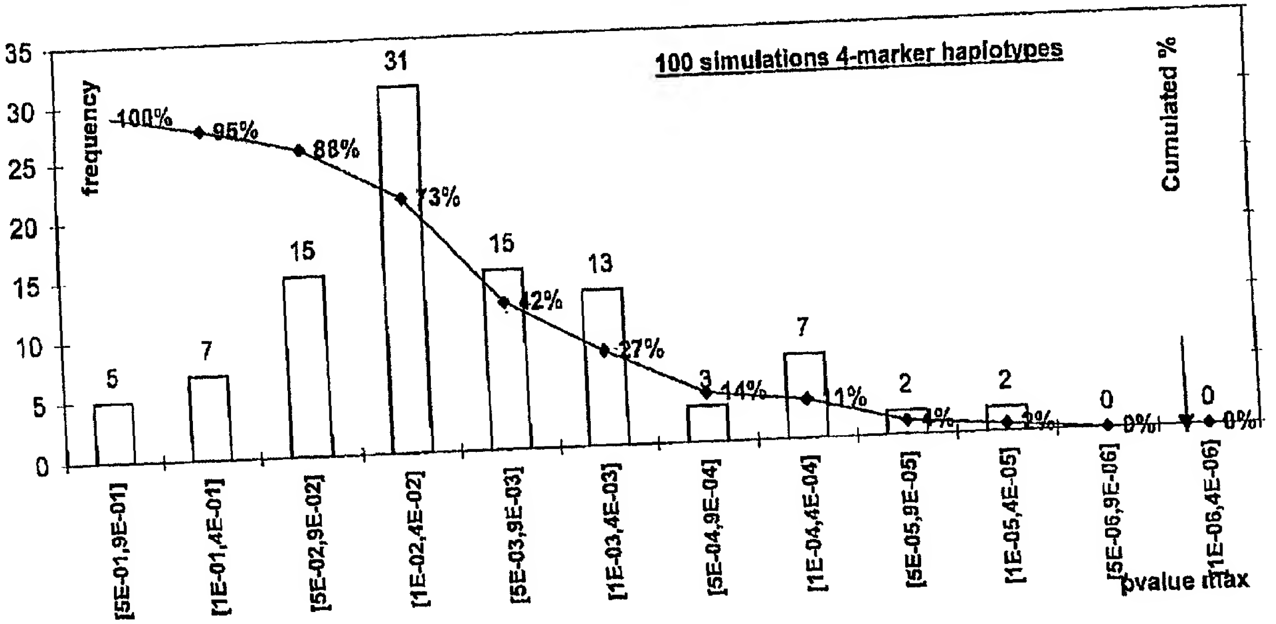


Figure 8

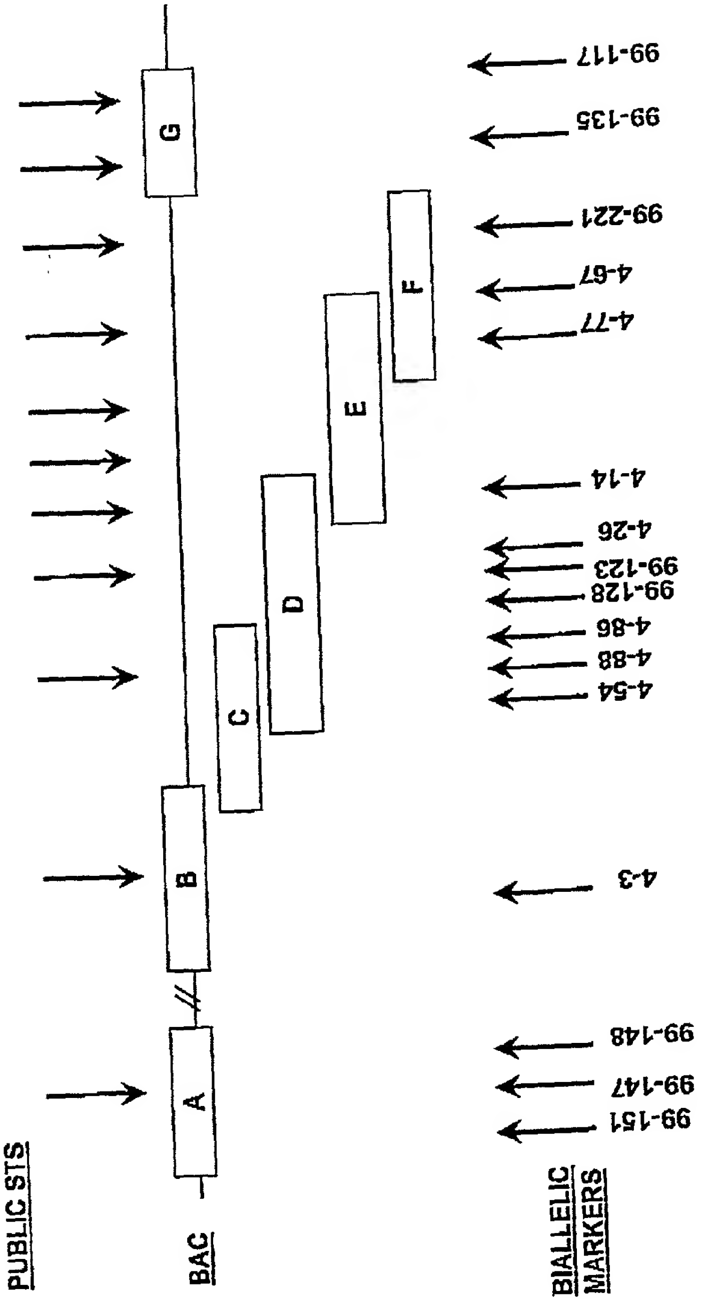

PUBLIC STS
BAC
BIALLELIC MARKERS
A
B
C
D
E
F
G
99-151
99-147
99-148
4-3
4-54
4-88
4-86
99-128
99-123
4-26
4-14
4-77
4-67
99-221
99-135
99-117

Figure 9

PROSTATE CANCER ASSOCIATION STUDIES (FIRST SCREENING)

Population Sample size	PROSTATE CANCER CASES = 112	NON AFFECTED CONTROLS=76
Population Characteristics	35 sporadic cases + 77 familial cases	> 65 years PSA<4

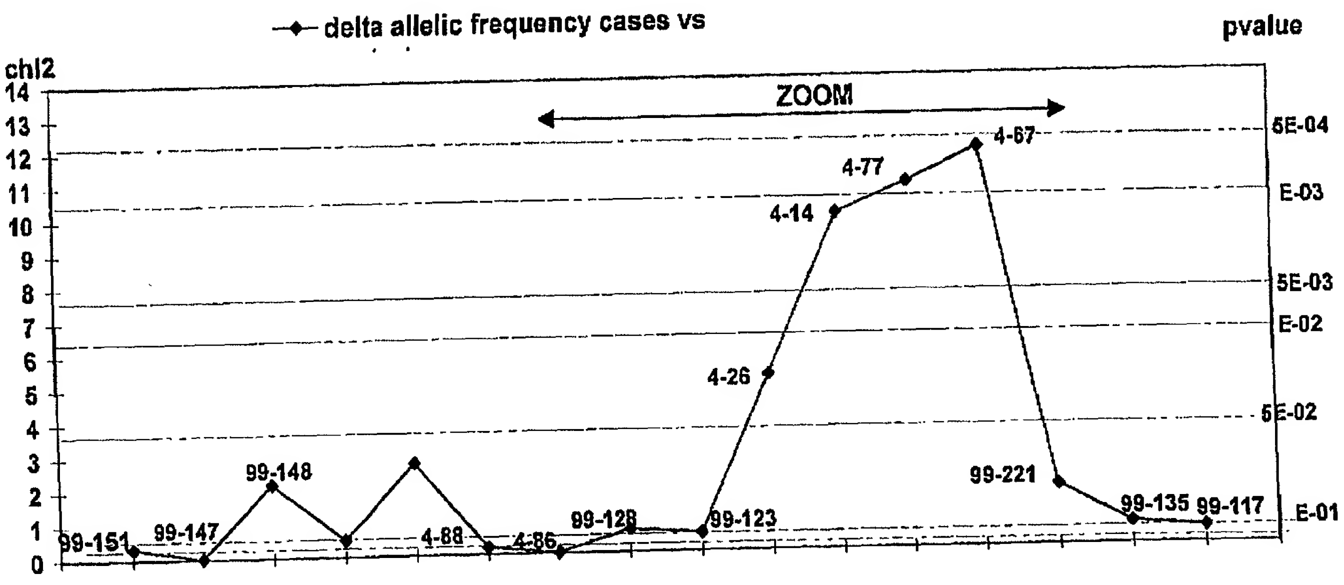


Figure 10

PROSTATE CANCER ASSOCIATION STUDIES (ZOOM)

	PROSTATE CANCER CASES (185)	NON-AFFECTED CONTROLS (104)
characteristics of populations	47 sporadic cases + 138 familial cases	> 65 years PSA<4

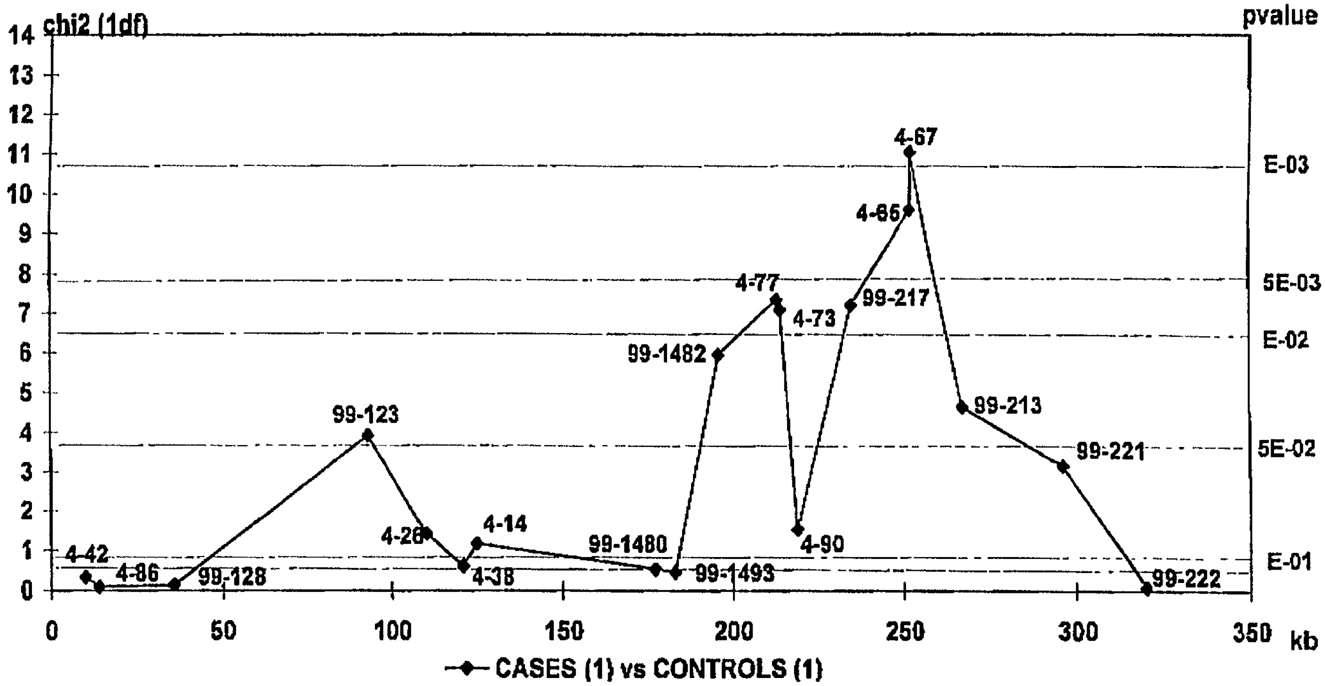


Figure 11

PROSTATE CANCER HAPLOTYPE FREQUENCY ANALYSIS

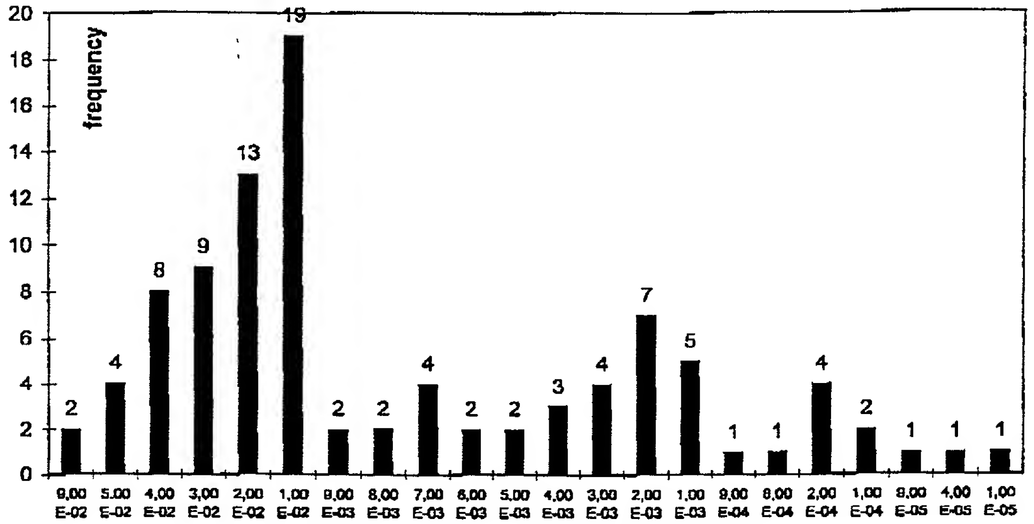
	PROSTATE CANCER CASES (281)	NON-AFFECTED CONTROLS (130)
characteristics of populations	143 sporadic cases + 138 familial cases	> 65 years PSA<4

markers	99-123	4-26	4-14	4-77	99-217	4-67	99-213	99-221	99-135	haplotype frequencies		relative risk	pvalue
bacs	H0287B09	B0189E08		B0463F01					B0725B12				
genes				<———PG1———>									
p value	2,00E-01	1,00E-01	1,00E-01	2,00E-02	2,00E-02	6,00E-04	9,00E-02	7,00E-01	2,00E-01	cases	controls		
haplotype 8 >304kb<	C	A	C	G	T	T	C	A	A	0,075	0,018	4,42	9,00E-04 ***
haplotype 7 >286kb<		A	C	G	T	T	C	A	A	0,095	0,016	6,46	6,00E-05 ****
haplotype 6 <186kb>		A	C	G	T	T	C	A		0,116	0,019	6,78	1,00E-05 *****
haplotype 5 <171kb>			C	G	T	T	C	A		0,117	0,013	10,06	9,00E-07 ******
haplotype 4 <83kb>				G	T	T	C	A		0,117	0,025	5,17	2,00E-05 *****
haplotype 3.1 <54kb>					T	T	C	A		0,117	0,027	4,78	2,00E-05 *****
haplotype 3.2 <54kb>				G	T	T	C			0,222	0,109	2,33	4,00E-05 *****
haplotype 2.2 <39kb>				G	T	T				0,251	0,134	2,17	2,00E-04 ****
haplotype 2 <32kb>					T	T	C			0,226	0,112	2,32	1,00E-04 ***
haplotype 1.1 <17 kb>					T	T				0,256	0,146	2,01	3,00E-04 ****
haplotype 1.2 <15 kb>						T	C			0,233	0,129	2,05	6,00E-04 ***

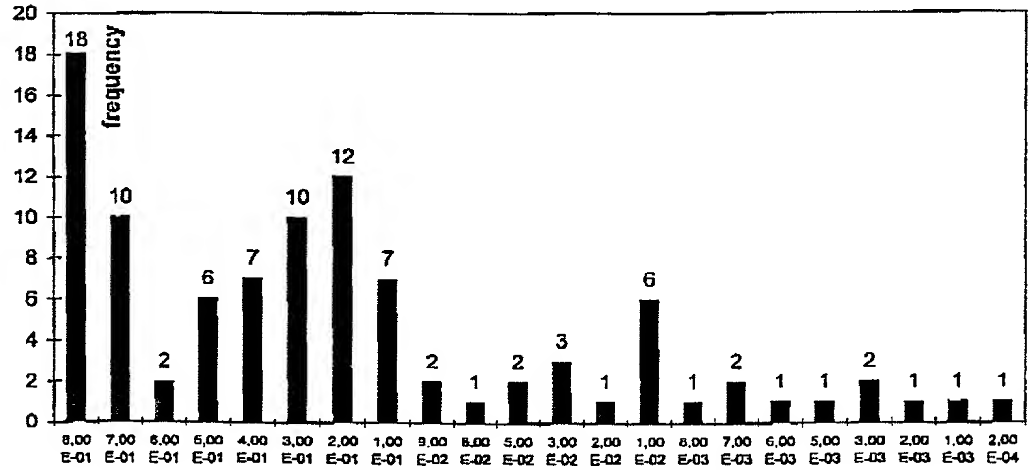
Figure 12

PROSTATE CANCER HAPLOTYPE SIMULATIONS (100 ITERATIONS)

							haplotype frequencies		relative risk	pvalue
markers	4-14	4-77	99-217	4-67	99-213	99-221	cases	controls		
haplotype	C	G	T	T	G	A	0,117	0,013	10,06	9,00E-07



pvalue max of haplotypes for 100 simulations



pvalue of haplotype CGTTGA for 100 simulations

Figure 13

AVERAGE LD PATTERN GENOMIC HETEROGENEITY

Recombination rate	Lower A	Higher B
Nb markers	89	69
All SNP	0.61 (749)	0.42 (1190)
Rare < 0.2 Rare vs rare	0.75 (65)	0.17 (158)
Frequent > 0.2 Frequent vs frequent	0.51 (410)	0.49 (544)
Rare vs frequent	0.72 (274)	0.41 (488)

FIGURE 14

Exonic/nonexonic LD

	Nb pairs	Average intermarker distance	Average LD
Exonic SNPs	36	26 kb	0.65±0.021
Non exonic SNPs	60	36 kb	0.48±0.018
Exonic/Non exonic	96	32 kb	**0.60±0.015**

FIGURE 15

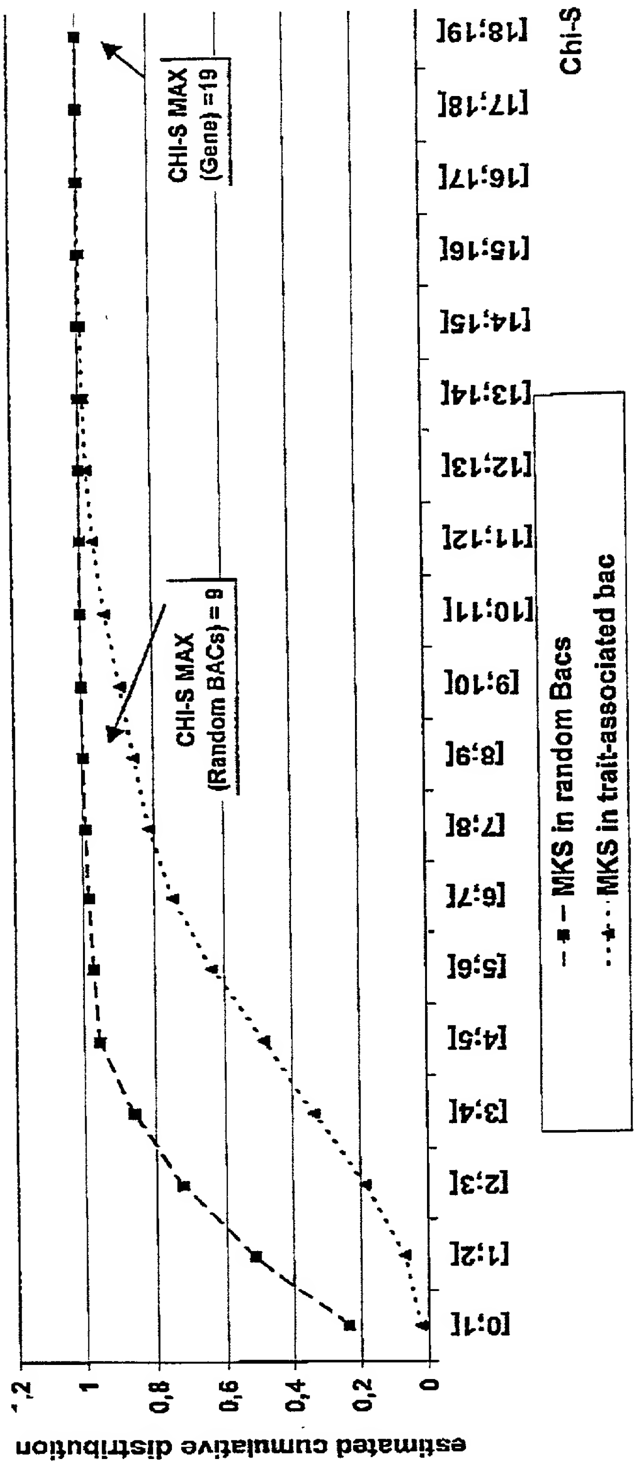

estimated cumulative distribution
1,2
1
0,8
0,6
0,4
0,2
0
[0;1]
[1;2]
[2;3]
[3;4]
[4;5]
[5;6]
[6;7]
[7;8]
[8;9]
[9;10]
[10;11]
[11;12]
[12;13]
[13;14]
[14;15]
[15;16]
[16;17]
[17;18]
[18;19]
Chi-S
CHI-S MAX (Random BACs) = 9
CHI-S MAX (Gene) =19
– ■ – MKS in random Bacs
··▲·· MKS in trait-associated bac

FIGURE 16 A

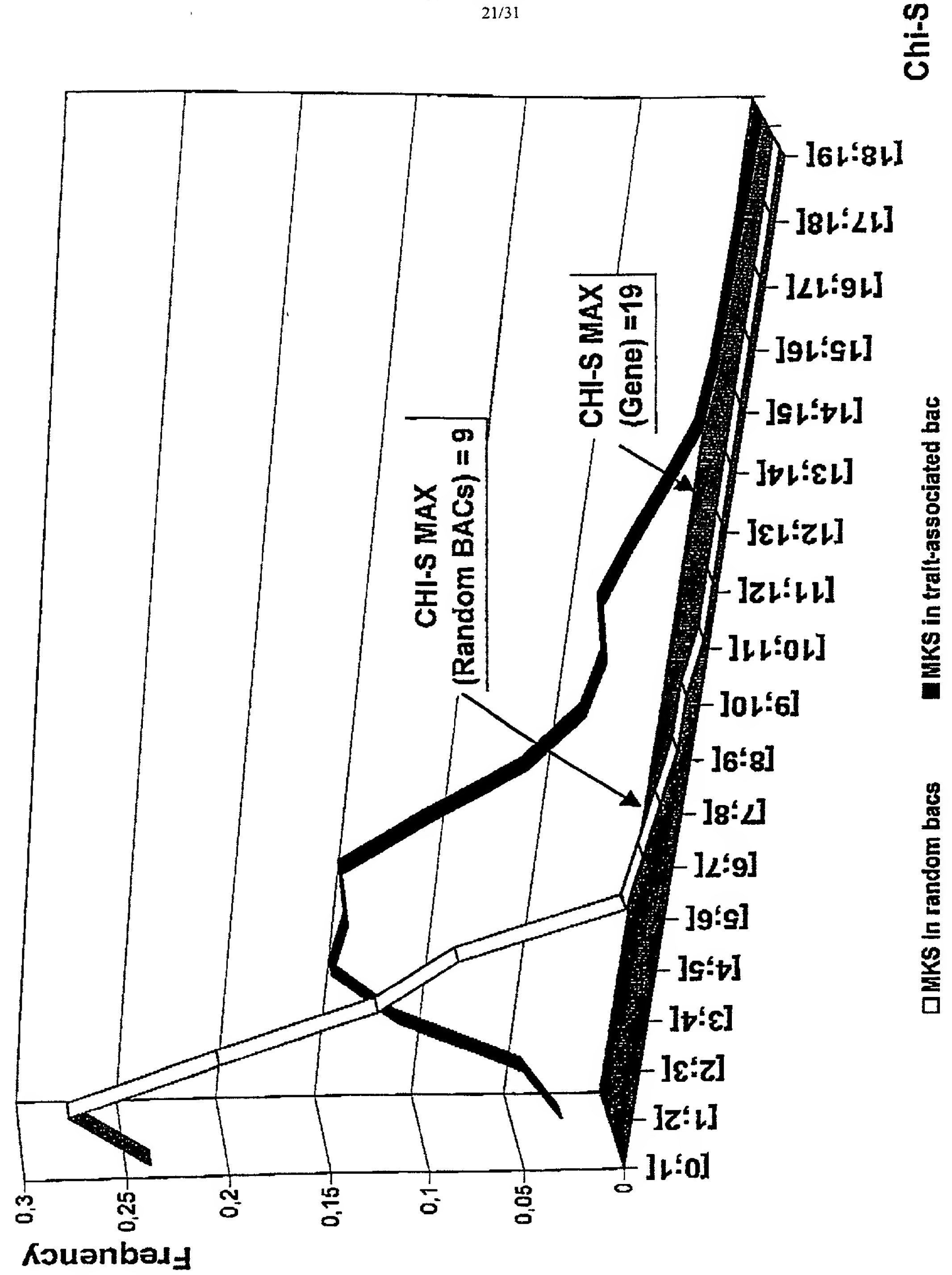

Chi-S
Frequency
0,3
0,25
0,2
0,15
0,1
0,05
0
]0;1]
]1;2]
]2;3]
]3;4]
]4;5]
]5;6]
]6;7]
]7;8]
]8;9]
]9;10]
]10;11]
]11;12]
]12;13]
]13;14]
]14;15]
]15;16]
]16;17]
]17;18]
]18;19]
CHI-S MAX (Random BACs) = 9
CHI-S MAX (Gene) =19
□MKS in random bacs
■MKS in trait-associated bac

FIGURE 16B

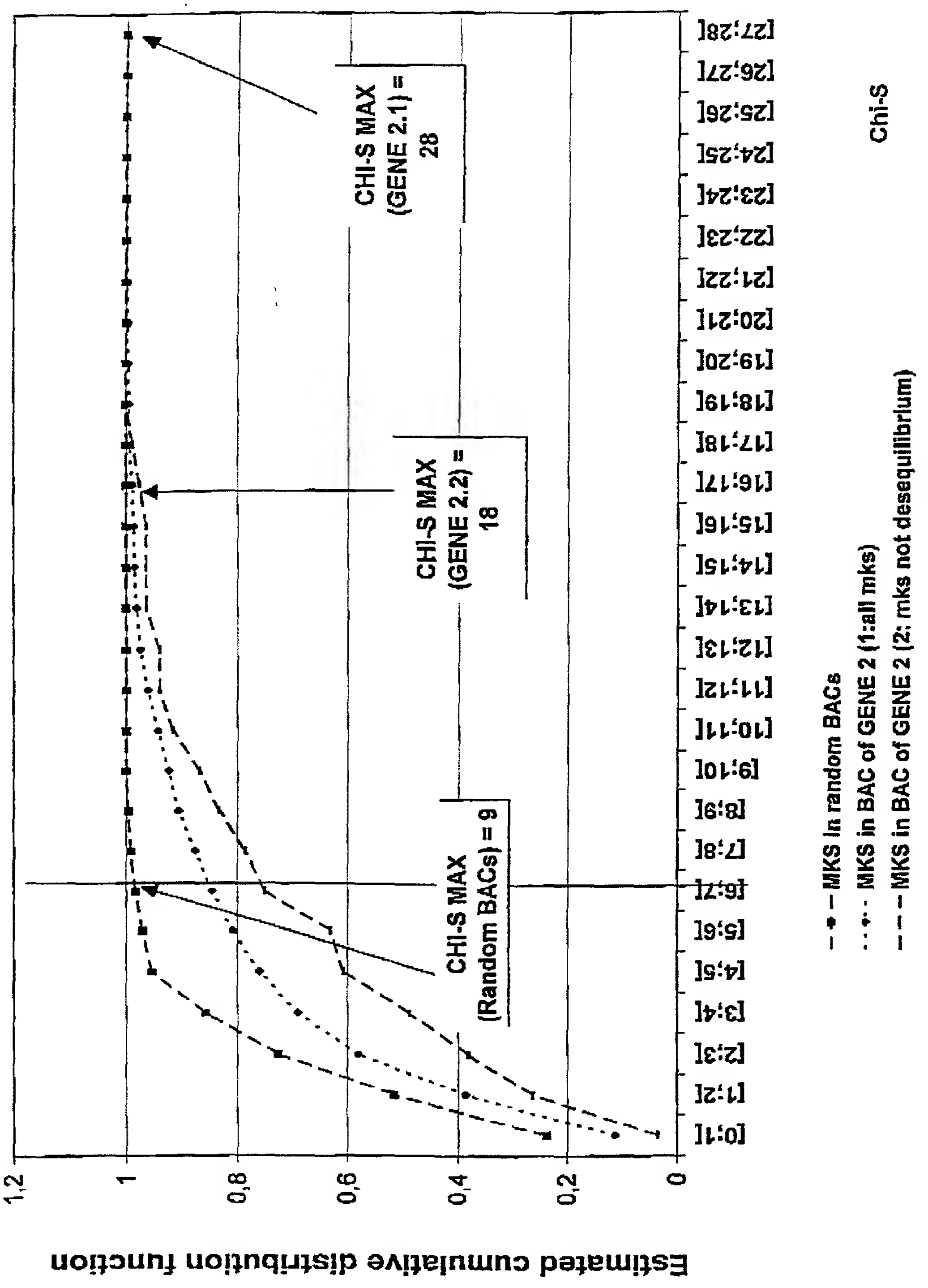

Chi-S
Estimated cumulative distribution function
0
0,2
0,4
0,6
0,8
1
1,2
[0;1]
[1;2]
[2;3]
[3;4]
[4;5]
[5;6]
[6;7]
[7;8]
[8;9]
[9;10]
[10;11]
[11;12]
[12;13]
[13;14]
[14;15]
[15;16]
[16;17]
[17;18]
[18;19]
[19;20]
[20;21]
[21;22]
[22;23]
[23;24]
[24;25]
[25;26]
[26;27]
[27;28]
CHI-S MAX (Random BACs) = 9
CHI-S MAX (GENE 2.2) = 18
CHI-S MAX (GENE 2.1) = 28
MKS in random BACs
MKS in BAC of GENE 2 (1:all mks)
MKS in BAC of GENE 2 (2: mks not desequilibrium)

FIGURE 17A

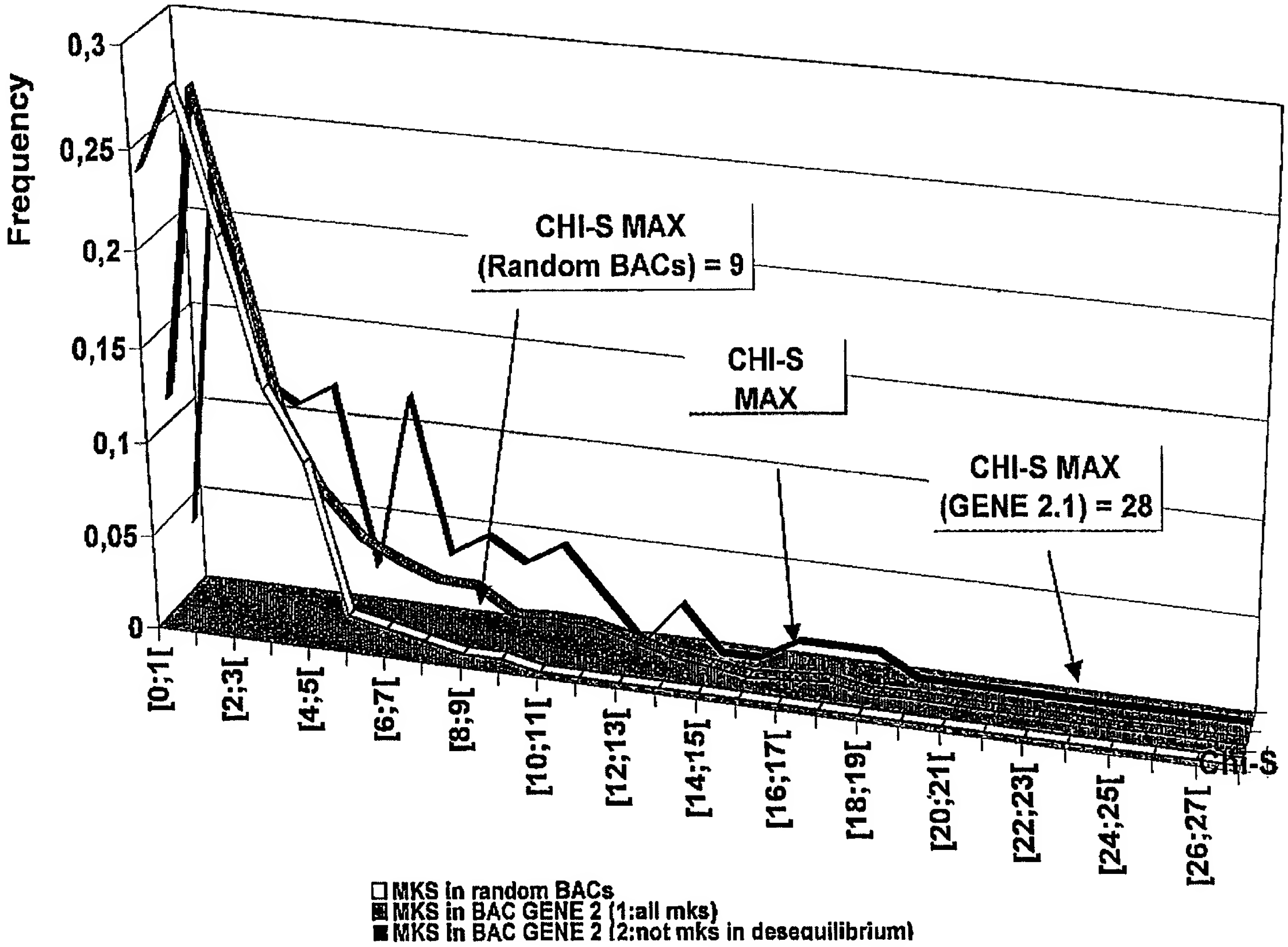

Frequency
0,3
0,25
0,2
0,15
0,1
0,05
0
[0;1[
[2;3[
[4;5[
[6;7[
[8;9[
[10;11[
[12;13[
[14;15[
[16;17[
[18;19[
[20;21[
[22;23[
[24;25[
[26;27[
CHI-S
CHI-S MAX
(Random BACs) = 9
CHI-S
MAX
CHI-S MAX
(GENE 2.1) = 28
☐ MKS in random BACs
▨ MKS in BAC GENE 2 (1:all mks)
■ MKS in BAC GENE 2 (2:not mks in desequilibrium)

FIGURE 17 B

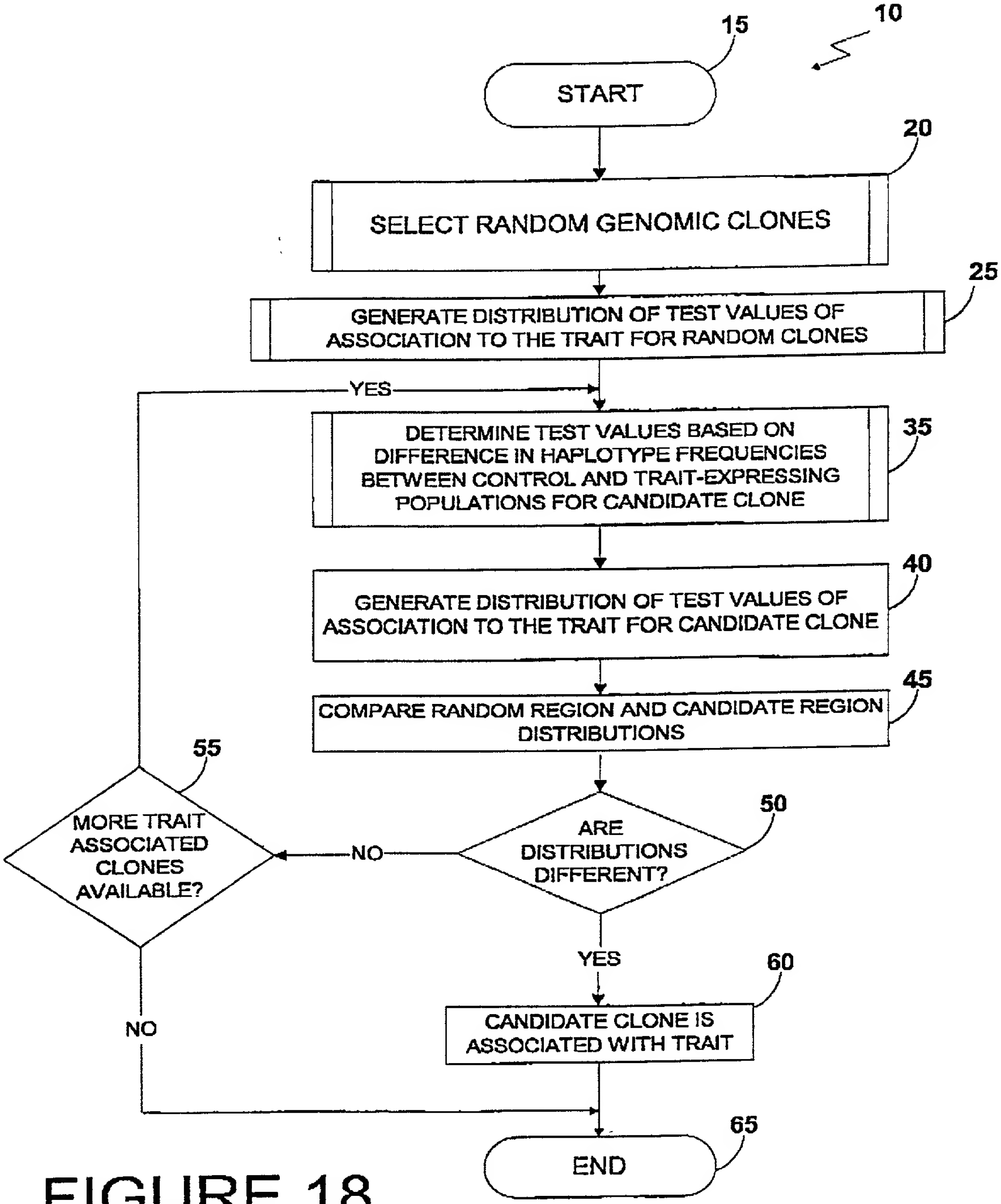

10
15
START
20
SELECT RANDOM GENOMIC CLONES
25
GENERATE DISTRIBUTION OF TEST VALUES OF ASSOCIATION TO THE TRAIT FOR RANDOM CLONES
YES
35
DETERMINE TEST VALUES BASED ON DIFFERENCE IN HAPLOTYPE FREQUENCIES BETWEEN CONTROL AND TRAIT-EXPRESSING POPULATIONS FOR CANDIDATE CLONE
40
GENERATE DISTRIBUTION OF TEST VALUES OF ASSOCIATION TO THE TRAIT FOR CANDIDATE CLONE
45
COMPARE RANDOM REGION AND CANDIDATE REGION DISTRIBUTIONS
55
MORE TRAIT ASSOCIATED CLONES AVAILABLE?
NO
ARE DISTRIBUTIONS DIFFERENT?
50
YES
60
CANDIDATE CLONE IS ASSOCIATED WITH TRAIT
NO
65
END

FIGURE 18

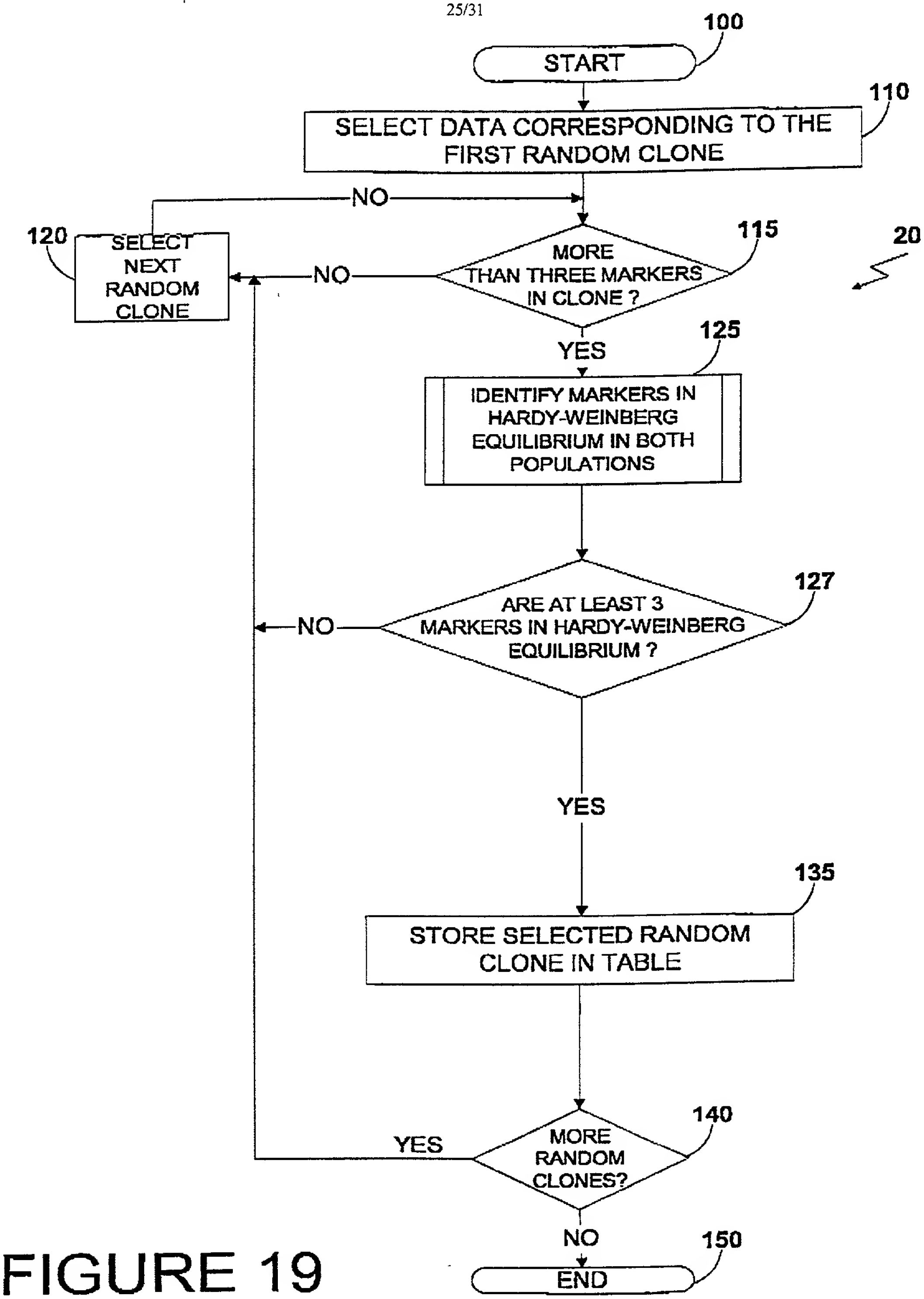

100
START
110
SELECT DATA CORRESPONDING TO THE FIRST RANDOM CLONE
NO
120
SELECT NEXT RANDOM CLONE
NO
MORE THAN THREE MARKERS IN CLONE ?
115
20
YES
125
IDENTIFY MARKERS IN HARDY-WEINBERG EQUILIBRIUM IN BOTH POPULATIONS
127
NO
ARE AT LEAST 3 MARKERS IN HARDY-WEINBERG EQUILIBRIUM ?
YES
135
STORE SELECTED RANDOM CLONE IN TABLE
140
YES
MORE RANDOM CLONES?
NO
150
END

FIGURE 19

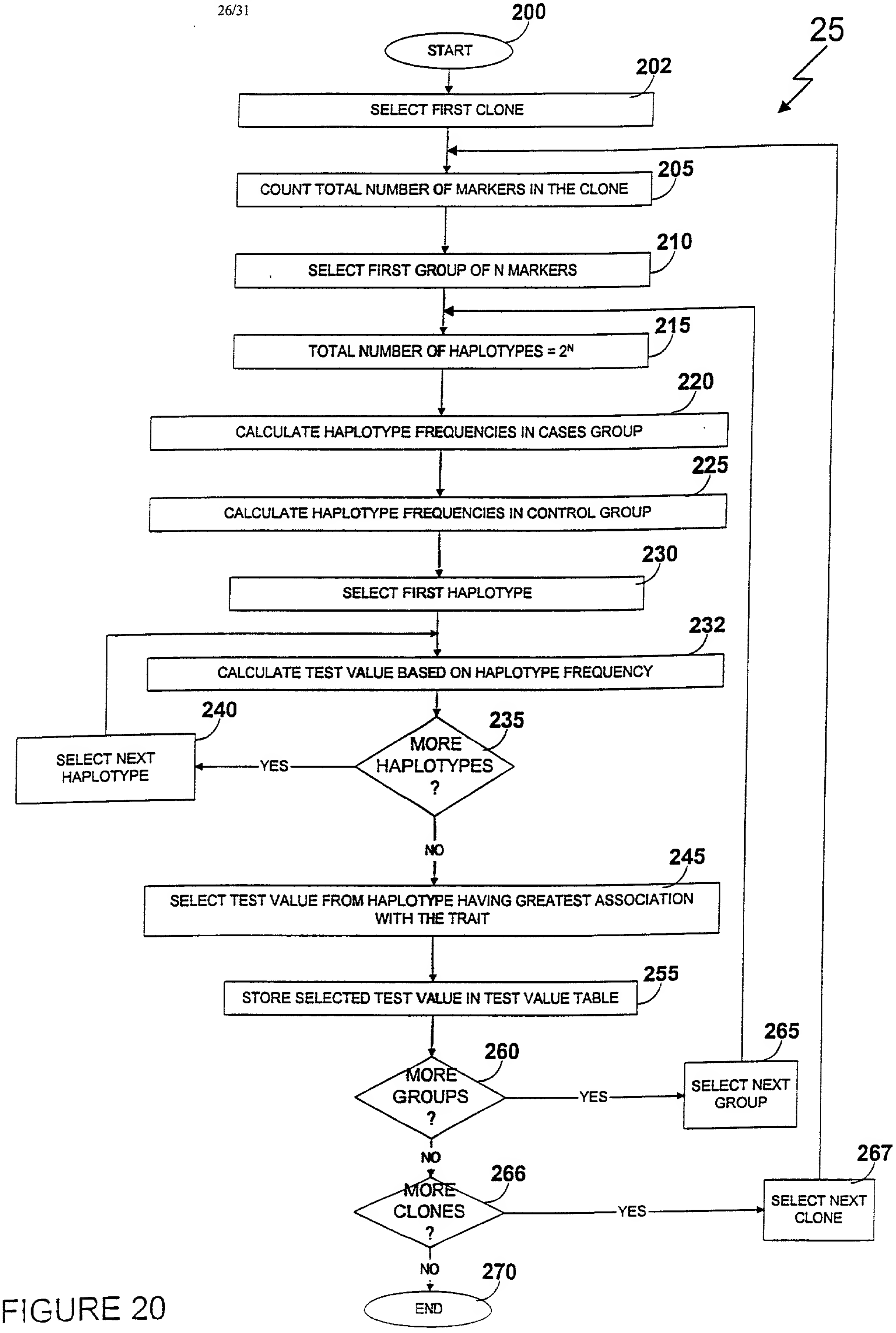

25
200
START
202
SELECT FIRST CLONE
205
COUNT TOTAL NUMBER OF MARKERS IN THE CLONE
210
SELECT FIRST GROUP OF N MARKERS
215
TOTAL NUMBER OF HAPLOTYPES = 2^N
220
CALCULATE HAPLOTYPE FREQUENCIES IN CASES GROUP
225
CALCULATE HAPLOTYPE FREQUENCIES IN CONTROL GROUP
230
SELECT FIRST HAPLOTYPE
232
CALCULATE TEST VALUE BASED ON HAPLOTYPE FREQUENCY
240
SELECT NEXT HAPLOTYPE
YES
235
MORE HAPLOTYPES ?
NO
245
SELECT TEST VALUE FROM HAPLOTYPE HAVING GREATEST ASSOCIATION WITH THE TRAIT
255
STORE SELECTED TEST VALUE IN TEST VALUE TABLE
260
MORE GROUPS ?
YES
265
SELECT NEXT GROUP
NO
266
MORE CLONES ?
YES
267
SELECT NEXT CLONE
NO
270
END

FIGURE 20

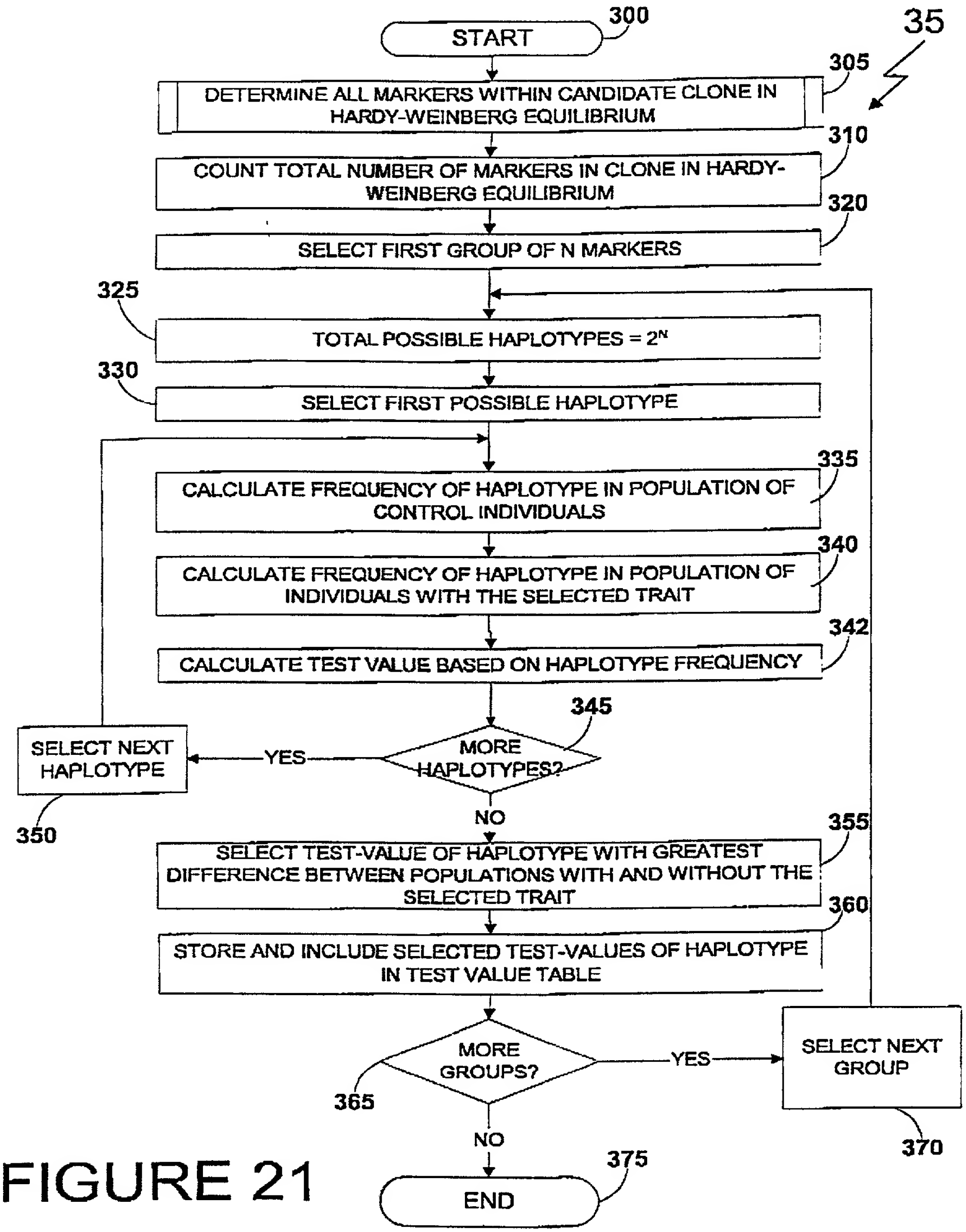

35
300
START
305
DETERMINE ALL MARKERS WITHIN CANDIDATE CLONE IN HARDY-WEINBERG EQUILIBRIUM
310
COUNT TOTAL NUMBER OF MARKERS IN CLONE IN HARDY-WEINBERG EQUILIBRIUM
320
SELECT FIRST GROUP OF N MARKERS
325
TOTAL POSSIBLE HAPLOTYPES = 2^N
330
SELECT FIRST POSSIBLE HAPLOTYPE
335
CALCULATE FREQUENCY OF HAPLOTYPE IN POPULATION OF CONTROL INDIVIDUALS
340
CALCULATE FREQUENCY OF HAPLOTYPE IN POPULATION OF INDIVIDUALS WITH THE SELECTED TRAIT
342
CALCULATE TEST VALUE BASED ON HAPLOTYPE FREQUENCY
345
MORE HAPLOTYPES?
YES
SELECT NEXT HAPLOTYPE
350
NO
355
SELECT TEST-VALUE OF HAPLOTYPE WITH GREATEST DIFFERENCE BETWEEN POPULATIONS WITH AND WITHOUT THE SELECTED TRAIT
360
STORE AND INCLUDE SELECTED TEST-VALUES OF HAPLOTYPE IN TEST VALUE TABLE
MORE GROUPS?
365
YES
SELECT NEXT GROUP
370
NO
375
END

FIGURE 21

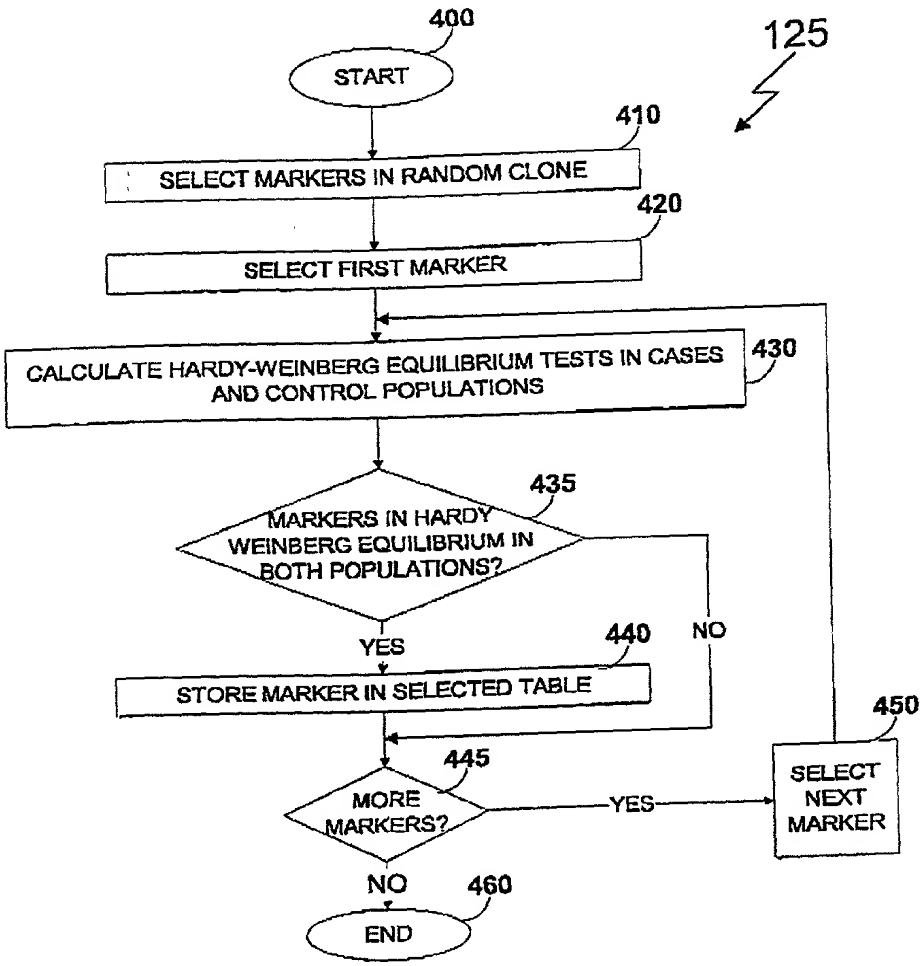

125
400
START
410
SELECT MARKERS IN RANDOM CLONE
420
SELECT FIRST MARKER
430
CALCULATE HARDY-WEINBERG EQUILIBRIUM TESTS IN CASES AND CONTROL POPULATIONS
435
MARKERS IN HARDY WEINBERG EQUILIBRIUM IN BOTH POPULATIONS?
YES
NO
440
STORE MARKER IN SELECTED TABLE
445
MORE MARKERS?
YES
450
SELECT NEXT MARKER
NO
460
END

FIGURE 22

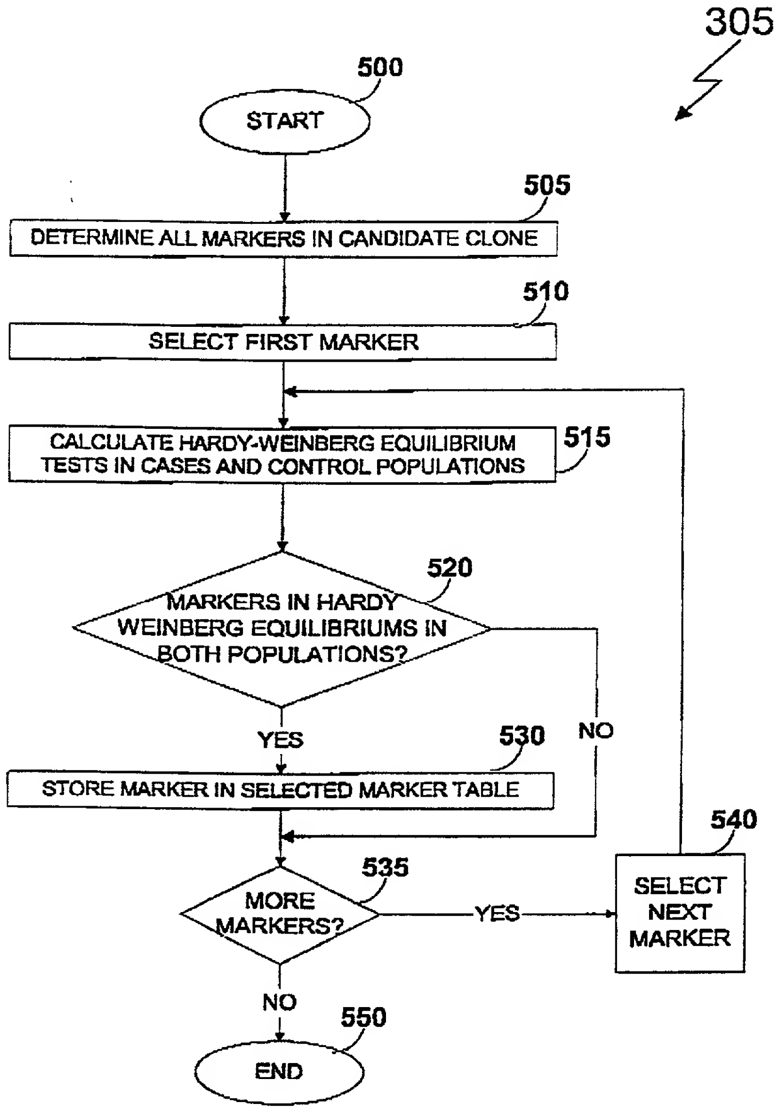

305
500
START
505
DETERMINE ALL MARKERS IN CANDIDATE CLONE
510
SELECT FIRST MARKER
CALCULATE HARDY-WEINBERG EQUILIBRIUM TESTS IN CASES AND CONTROL POPULATIONS
515
520
MARKERS IN HARDY WEINBERG EQUILIBRIUMS IN BOTH POPULATIONS?
YES
530
NO
STORE MARKER IN SELECTED MARKER TABLE
540
535
MORE MARKERS?
YES
SELECT NEXT MARKER
NO
550
END

FIGURE 23

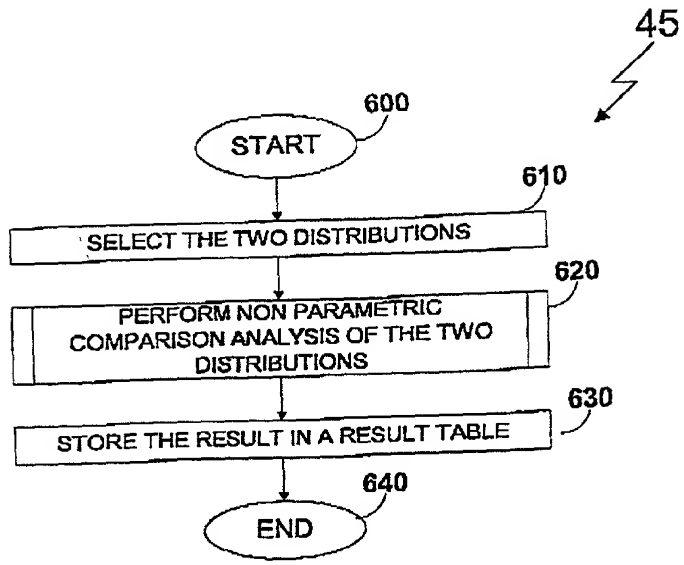

45
600
START
610
SELECT THE TWO DISTRIBUTIONS
620
PERFORM NON PARAMETRIC COMPARISON ANALYSIS OF THE TWO DISTRIBUTIONS
630
STORE THE RESULT IN A RESULT TABLE
640
END

FIGURE 24

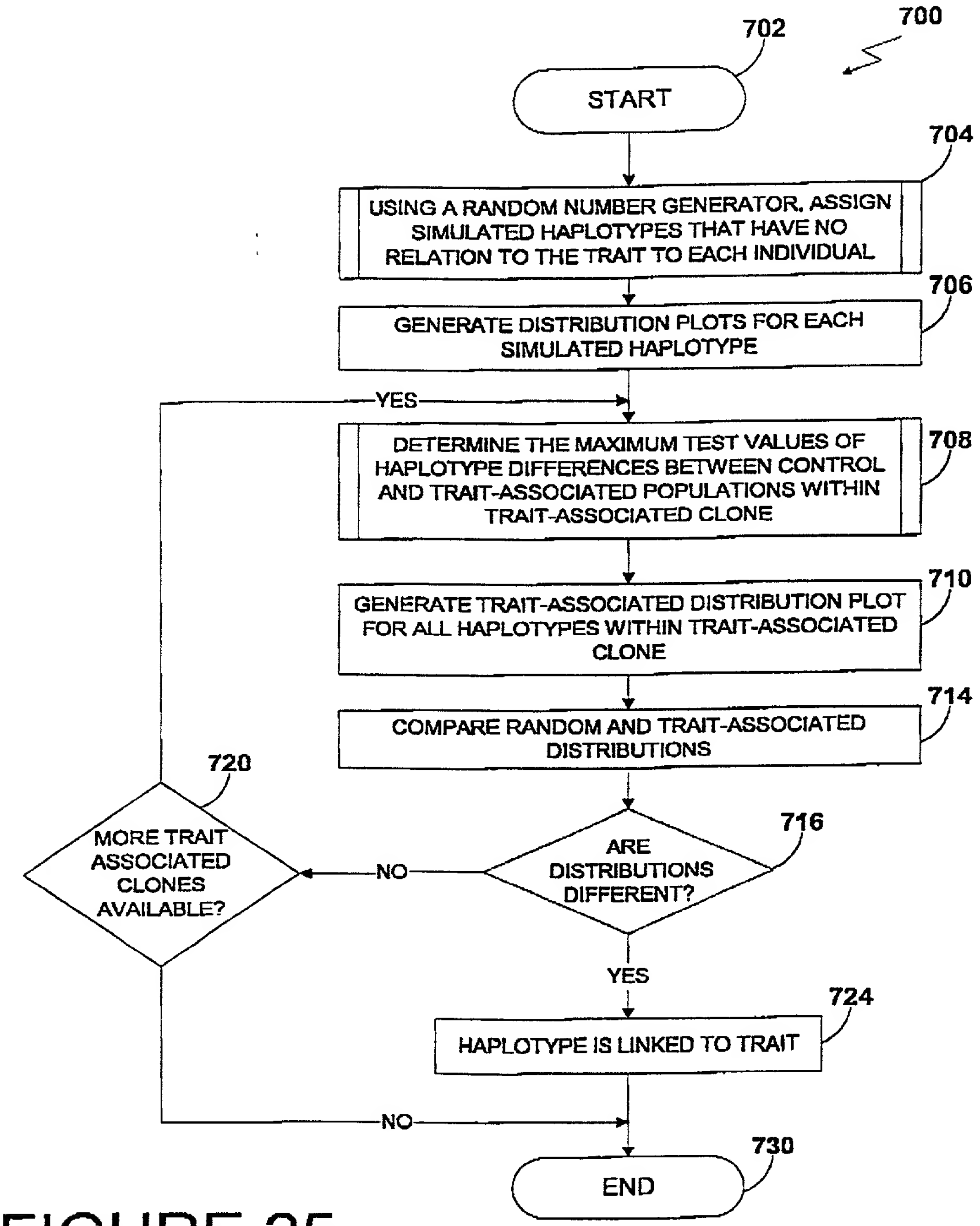

700
702
START
704
USING A RANDOM NUMBER GENERATOR, ASSIGN SIMULATED HAPLOTYPES THAT HAVE NO RELATION TO THE TRAIT TO EACH INDIVIDUAL
706
GENERATE DISTRIBUTION PLOTS FOR EACH SIMULATED HAPLOTYPE
YES
708
DETERMINE THE MAXIMUM TEST VALUES OF HAPLOTYPE DIFFERENCES BETWEEN CONTROL AND TRAIT-ASSOCIATED POPULATIONS WITHIN TRAIT-ASSOCIATED CLONE
710
GENERATE TRAIT-ASSOCIATED DISTRIBUTION PLOT FOR ALL HAPLOTYPES WITHIN TRAIT-ASSOCIATED CLONE
714
COMPARE RANDOM AND TRAIT-ASSOCIATED DISTRIBUTIONS
720
MORE TRAIT ASSOCIATED CLONES AVAILABLE?
NO
716
ARE DISTRIBUTIONS DIFFERENT?
YES
724
HAPLOTYPE IS LINKED TO TRAIT
NO
730
END

FIGURE 25